bio-techne®
R&D SYSTEMS

DuoSet™ Ancillary Reagent Kit 1

For 96-well (DY007C) and 384-well (DY007C/384) Microplates

Online Use Only

MATERIALS PROVIDED & STORAGE CONDITIONS

Store the unopened kit at 2-8 °C. Do not use past kit expiration date.

PART	PART #	DESCRIPTION	STORAGE OF OPENED/DILUTED MATERIAL
ELISA Plate-Coating Buffer	896036	60 mL of sterile-filtered 1X PBS for use as a plate-coating buffer in ELISA development kits. Contains no preservatives.	May be stored for up to 3 months at 2-8 °C when handled aseptically.*
Reagent Diluent Concentrate 1	840149	21 mL of a buffered protein base.	May be stored for up to 60 days at 2-8 °C.* *Use within 8 hours of dilution. Prepare fresh for each assay.*
Reagent Diluent Concentrate 3 (5X)	895956	2 vials (21 mL/vial) of a 25% Tween® 20 solution in PBS. *Use as the blocking buffer.*	May be stored for up to 1 month at 2-8 °C.* *Use and discard diluted reagent diluent. Prepare fresh for each assay.*
ELISA Stop Solution**	642827	5 vials (12 mL/vial) of Methanesulfonic acid.	May be stored for up to 1 month at 2-8 °C.*
TMB ELISA Substrate	642736	5 vials (12 mL/vial) of substrate.	
Wash Buffer Concentrate	895003	7 vials (21 mL/vial) of a concentrated solution of buffered surfactant with preservative. *May turn yellow over time.*	
20X PBS	896034	21 mL of concentrated PBS.	
Microplates	**DY007C:** 640026 **DY007C/384:** 608616	5 clear 96-well high-binding flat-bottom plates. 5 flat-bottom 384-well polystyrene plates.	Cover any unused plates with the ELISA Plate Sealers provided.
ELISA Plate Sealers	640197	24 adhesive strips	

* Provided this is within the expiration date of the kit.

**In both formats, 96- and 384-well microplates, the addition of Stop Solution halts the activity of horseradish peroxidase on the TMB substrate. If the wells appear green or if the color change is uneven, gently tap the plate to ensure thorough mixing.

- For 96-well microplate format (DY007C): Add 100 µL/well.
- For 384-well microplate format (DY007C/384): Add 25 µL/well.

REAGENT PREPARATION

Bring all reagents to room temperature before use.

Reagent preparations listed below are sufficient for one 384-well plate. Adjust volumes accordingly.

1X PBS: Add 2 mL of 20X PBS to 38 mL deionized or distilled water to prepare 40 mL 1X PBS.

Reagent Diluent 3 (diluted 1:5) - Add 8 mL of Reagent Diluent Concentrate 3 (5X) to 32 mL of 1X PBS to prepare 40 mL of Reagent Diluent 3 (diluted 1:5) before use.

Reagent Diluent 1 (diluted) - Add 1.4 mL of Reagent Diluent Concentrate 1 to 98.6 mL of 1X Wash Buffer to prepare 100 mL of Reagent Diluent 1 (diluted).

TMB ELISA Substrate - Protect from light.

1X Wash Buffer - If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have gone into solution. Add 28 mL of Wash Buffer Concentrate to 672 mL of deionized or distilled water to prepare 700 mL of 1X Wash Buffer.

bio-techne®
Global bio-techne.com info@bio-techne.com TEL +1 612 379 2956 **USA** TEL 800 343 7475 **Canada** TEL 855 668 8722
Europe | Middle East | Africa TEL +44 (0)1235 529449 **China** TEL +86 (21) 52380373

SUGGESTED ELISA PROTOCOL

For 94-Well Plates (DY007C):

Protocol generally provided with the target-specific DuoSet™ kit. Follow guidance for diluents, dilution ranges, typical data, and troubleshooting. Standard protocol typically uses 100 µL per well. Refer to DuoSet insert and CofA for details.

For 384-Well Plates (DY007C/384):

Further optimization may be required.

Plate Preparation

1. Dilute the Capture Antibody to the working concentration in PBS without carrier protein. Immediately coat a 384-well microplate with 25 µL per well of the diluted Capture Antibody. Seal the plate and incubate overnight at room temperature.
2. Aspirate each well and wash with Wash Buffer, repeating the process two times for a total of three washes. Wash by filling each well with Wash Buffer (100 µL) using a squirt bottle, manifold dispenser, or autowasher. Complete removal of liquid at each step is essential for good performance. After the last wash, remove any remaining Wash Buffer by aspirating or by inverting the plate and blotting it against clean paper towels.
3. Block plates by adding 75 µL of Reagent Diluent to each well. Incubate at room temperature for a minimum of 1 hour.
4. Repeat the aspiration/wash as in step 2. The plates are now ready for sample addition.

Assay Procedure

1. Add 25 µL of standards or sample in Reagent Diluent, or an appropriate diluent, per well. Cover with an adhesive strip and incubate 2 hours at room temperature.
2. Repeat the aspiration/wash as in step 2 of the Plate Preparation.
3. Add 25 µL of the Detection Antibody, diluted in Reagent Diluent, to each well. Cover with a new adhesive strip and incubate 2 hours at room temperature.
4. Repeat the aspiration/wash as in step 2 of the Plate Preparation.
5. Add 25 µL of the working dilution of Streptavidin-HRP to each well. Cover the plate and incubate for 20 minutes at room temperature. **Avoid placing the plate in direct light.**
6. Repeat the aspiration/wash as in step 2.
7. Add 25 µL of Substrate Solution to each well. Incubate for 20 minutes at room temperature. Avoid placing the plate in direct light.
8. Add 25 µL of Stop Solution to each well. Gently tap the plate to ensure thorough mixing.
9. Determine the optical density of each well immediately, using a microplate reader set to 450 nm. If wavelength correction is available, set to 540 nm or 570 nm. If wavelength correction is not available, subtract readings at 540 nm or 570 nm from the readings at 450 nm. This subtraction will correct for optical imperfections in the plate. Readings made directly at 450 nm without correction may be higher and less accurate.

PRECAUTIONS

The Stop Solution provided with this kit is an acid solution.

Some components in this kit contain a preservative which may cause an allergic skin reaction. Avoid breathing mist.

Wear protective gloves, clothing, eye, and face protection. Wash hands thoroughly after handling. Refer to the SDS on our website prior to use.

TECHNICAL HINTS

- It is recommended that all standards and samples be assayed in triplicate.
- It is recommended that an automated liquid handling system is used with the 384-well microplate. Significant well-to-well variations can occur if an automated system is not used.
- Refer to the product datasheet and/or CofA for any target-specific information regarding recommended assay diluents, dilution ranges, typical data, and troubleshooting.
- It is important to ensure liquid fully coats the bottom of the plate at each step. This can be helped by spinning down the 384-well plate with a microplate centrifuge for 20 seconds at 850 x g.